fermentation

MDPI

Article

Potential of Endophytic Microorganisms in Fermentative Processes Using Agro-Industrial Waste as Substrates

Maria Terezinha Santos Leite Neta [1], Rafael Donizete Dutra Sandes [1], Mônica Silva De Jesus [1], Hannah Caroline Santos Araujo [1], Raquel Anne Ribeiro Dos Santos [2], Luciana Cristina Lins De Aquino Santana [3] and Narendra Narain [1,*]

[1] Laboratory of Flavor and Chromatographic Analysis, Federal University of Sergipe, Av. Marechal Rondon, s/n, Jardim Rosa Elze, São Cristóvão 49100-000, Sergipe, Brazil; terezinhaleite@gmail.com (M.T.S.L.N.); rafael.donizete.dutra@gmail.com (R.D.D.S.); monicasj.sst@gmail.com (M.S.D.J.); hcarol197@gmail.com (H.C.S.A.)

[2] Federal Institute of Education, Science and Technology of Sergipe, Rod. BR 101, Km 96, s/n, Quissamã, São Cristóvão 49100-000, Sergipe, Brazil; eng.raquelanne@gmail.com

[3] Department of Food Technology, Laboratory of Food Microbiology and Bioengineering, Federal University of Sergipe, Av. Marechal Rondon, s/n, Jardim Rosa Elze, São Cristóvão 49100-000, Sergipe, Brazil; luciana.santana@academico.ufs.br

* Correspondence: narendra.narain@gmail.com; Tel.: +55-793-194-6514

Abstract: This study investigated the potential of endophytic microorganisms in fermentative processes using agro-industrial residues as substrates. The aim was to explore sustainable biotechnological methods for producing valuable compounds from waste. Endophytic microorganisms were isolated from strawberry, lychee, and tangerine and used in fermentative processes with passion fruit, guava, and pineapple residues. Key methods included isolating and screening potent aroma-producing microorganisms, identifying them using MALDI-TOF MS, and analyzing volatile compounds by GC-MS. The results identified 37 endophytic microorganisms, with *Kloeckera apiculata* presenting the greatest aromatic potential. Fermentation with agro-industrial residues produced significant volatile compounds, identifying 27, 23, and 22 compounds from passion fruit, pineapple, and guava residues, respectively. The main conclusions highlighted the high production of ethyl acetate and 2-phenylethyl acetate, which contributed to fruity and floral aromas. The novelty of this study lies in the use of endophytic microorganisms isolated from tropical fruits to biotransform agro-industrial waste into high-value aromatic compounds, offering economic and environmental benefits. This research is significant, as it proposes a sustainable approach to valorize waste and produce natural aroma compounds through biotechnological processes.

Keywords: fermentation; volatile compounds; *Kloeckera apiculata*; sensory analysis; GC-MS analysis; MALDI-TOF MS identification

check for updates

Citation: Leite Neta, M.T.S.; Sandes, R.D.D.; De Jesus, M.S.; Araujo, H.C.S.; Dos Santos, R.A.R.; Santana, L.C.L.D.A.; Narain, N. Potential of Endophytic Microorganisms in Fermentative Processes Using Agro-Industrial Waste as Substrates. *Fermentation* **2024**, *10*, 430. https://doi.org/10.3390/fermentation10080430

Academic Editors: Fernanda Cortez Lopes and Marilene Henning Vainstein

Received: 19 June 2024
Revised: 5 August 2024
Accepted: 15 August 2024
Published: 17 August 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The production of aroma compounds through biotechnological processes has significantly increased in recent years. This interest is partly due to the commercial appeal of these products, which are considered "natural" and "healthy" [1,2], as well as the responsible towards environmental issues, resulting in the development of cleaner processes. This helps the industry to adapt its processes and products to the latest global trends. Compounds classified as natural can be obtained through enzymatic or fermentative methods from organic material, whereas conventional production involves chemical processes [3,4].

The use of microorganisms in aroma production has been growing and improving over the years. Currently, around 100 aromatic compounds are produced industrially through fermentation by microorganisms [5]. Examples of these include the production of β-ionone, β-damascone, and pseudoionone using submerged fermentation in a synthetic medium

with microorganisms isolated from flowers [6]. Aggelopoulos et al. [7] obtained esters, alcohols, ketones, and terpenes through solid-state fermentation using whey, molasses, beer, orange, and potato residues with *Kluveromyces marxianus* and *Saccharomyces cerevisiae*.

Among the microorganisms used in fermentation, endophytes stand out, isolated from plant tissues with disinfected surfaces [8–10]. These microorganisms, isolated from different parts of plants such as flowers, fruits, leaves, stems, roots, and seeds [10–13], have the ability to live internally without causing any visible damage [14,15].

These microorganisms, which are composed mainly of fungi and bacteria, positively or neutrally interact with host plants without causing harm, establishing symbiotic and/or mutualistic relationships [13,14]. Several studies highlight the importance of endophytes as protective agents against attacks by microorganisms, insects, and herbivores, as well as their ability to produce phytohormones, enzymes, and other chemical compounds. The use of these microorganisms in the biotechnology industry has grown due to their biochemical versatility and genetic diversity.

The isolation of endophytic microorganisms, especially from fruit trees, has been gaining prominence in the literature. Rodrigues et al. [16] isolated approximately 13 endophytic fungi from leaves and stems of the cajazeira tree, with *Colletotrichum gloeosporioides*, *Guignardia* sp., and *Phomopsis* sp. being noteworthy. In banana leaves, Pereira et al. [17] identified 16 endophytic fungi, the most common being *Xylaria* sp., *Colletotrichum musae*, and *Cordana musae*. Uenojo and Pastore [18] isolated 104 endophytic microorganisms from coffee beans, coffee washing water, coffee plant leaves, coffee plantation soil, and fruits such as banana, tangerine, orange, melon, papaya, guava, grape, and apricot, seeking microorganisms efficient in the production of pectic substances.

Due to their biochemical versatility and genetic diversity, the application of these endophytic microorganisms has been prominent in biotechnological processes for aroma production. Additionally, the use of agro-industrial residues as a nutrient source for these microorganisms represents a strategy to reduce costs.

With one of the world's largest economies based on agriculture, Brazil has a significant production of agricultural materials, generating a vast amount of residues [19]. Recently, interest in reusing these residues has grown, enabling their application as a nutritional source in various bioprocesses, aiming to obtain high value-added chemical products such as ethanol, proteins, enzymes, organic acids, amino acids, biologically active secondary metabolites, and aromatic compounds [19,20].

The production of aromas through biotechnological processes can occur in two ways: synthesis of new compounds and biotransformation. In both cases, it is necessary for the precursor of the compounds to be present in the culture medium. Some precursors are found in agro-industrial residues such as peels, seeds, and bagasse, which become excellent nutrient sources for microbial growth.

As an example, the production of an intense fruity aroma by *Ceratocystis fimbriata* in a fermentation medium composed of wheat bran, sugarcane bagasse, and cassava bagasse was reported by Medeiros et al. [20]. The production of pineapple aroma in a medium containing a combination of apple pulp residues, cassava bagasse, and amaranth was reported by Pandey et al. [21]. Similarly, the production of volatile compounds such as acetaldehyde and 3-methyl butanol using tropical fruit residues as a fermentation medium for the growth of *Rhizopus oryzae* was described by Medeiros et al. [20].

With advancements and innovations in the field of biotechnology, new perspectives and possibilities are emerging regarding the use of agro-industrial residues. These residues have great potential as substrates in bioprocesses and in obtaining higher value-added products. Thus, the application of agro-industrial residues in bioprocesses represents a way to use alternative substrates and contribute to solving environmental pollution problems.

The present study aims to explore the potential of endophytic microorganisms in fermentative processes using agro-industrial waste as substrates for the production of sustainable aromatic compounds. By utilizing waste from passion fruit (*Passiflora edulis* Sims.), guava (*Psidium guajava* L.), and pineapple (*Ananas comosus* L. Merr.), the research

not only enhances the value of these by-products but also addresses environmental impact reduction. The use of endophytic microorganisms isolated from tropical fruits has proven effective in the biotransformation of waste into high-value products. The findings of this study are of great interest to researchers and biotechnology industry professionals due to the economic and ecological benefits of the developed processes.

2. Materials and Methods

2.1. Fruits

The fruits used for the isolation of endophytic microorganisms were strawberry (*Fragaria* × *ananassa* Duchesne.), lychee (*Litchi chinensis* Sonn.), and tangerine (*Citrus reticulata* Blanco.), which were purchased at CEASA (Supply Center of the State of Sergipe). The fruits were manually selected to exclude those with physical damage, insect attacks, or fungal infections.

2.2. Agro-Industrial Residues

The agro-industrial residues of passion fruit (*Passiflora edulis* Sims.), guava (*Psidium guajava* L.), and pineapple (*Ananas comosus* L. Merr.) were weighed and stored at −18 °C in a freezer, inside plastic packaging, for later analysis. The residues were prepared in powder form using a hammer and grinder (IKA, model A11 Basic) to facilitate the absorption of nutrients by the microorganism. The residues were donated by Pomar do Brasil Indústria e Comércio de Alimentos Ltd.a., located in Aracaju, SE, Brazil.

2.3. Isolation and Selection of Endophytic Microorganisms

2.3.1. Sanitization of Fruits

The fruits selected for isolation of endophytic microorganisms underwent prior sanitization treatment, described below. The collected samples were washed in running water with neutral soap to remove epiphytic fungi. Later, they were immersed in 70% (*v*/*v*) alcohol for 1 min to break the surface tension. Subsequently, they were immersed in 3% sodium hypochlorite (NaClO) for 3 min, promoting asepsis of the material. Finally, they were immersed again in 70% (*v*/*v*) alcohol to remove the excess sodium hypochlorite. As a negative control, plating of the last water used in asepsis of the samples was performed.

2.3.2. Isolation of Microorganisms

After the sanitization process, the fruits were subjected to the isolation method. The samples were washed in running water and cut with a sterile scalpel. Later, 10 mL of sterile water was added, and the samples were shaken for 1 min in a vortex. Subsequently, they were incubated in a BOD incubator for 7 d. After incubation, 0.1 mL of the supernatant was collected and diluted in tubes from 10^{-2} to 10^{-6}. The dilutions were used to prepare duplicate plates, which were incubated in a BOD incubator for another 7 d. Finally, the plates were streaked and analyzed in YM medium.

2.3.3. Selection of Endophytic Microorganisms Using Sensory Analysis

Selection of microorganisms with the potential for aroma production was carried out using sensory analysis according to the method adapted from Uenojo and Pastore [18]. For each isolated microorganism, a pre-inoculum was prepared, where three loops were added to 100 mL of YM medium and incubated in a shaker at 30 °C for 48 h at 150 rpm. After this period, 50 mL of a fermentation medium containing 5% fructose and 0.5% yeast extract was prepared. The strains were inoculated with 5 mL, and the flasks were incubated in a rotary shaker at 28 °C and 150 rpm for 72 h to evaluate the microorganism's ability to produce aroma using the same sensory panel.

A sensory team, composed of six panelists familiar with tropical fruit aromas and with prior experience on quantitative descriptive sensory teams for analyzing aromas and flavors of tropical fruits and derivatives, described and quantified the intensity of the

aromatic notes perceived in the fermented media compared to a non-inoculated control, using an unstructured scale. The results were analyzed by ANOVA and Tukey's mean test.

2.4. Identification of the Selected Microorganism

The microorganism was identified using a matrix-assisted laser desorption/ionization mass spectrometer (MALDI) from BRUKER Daltonics, model MALDI-TOF Microflex LT, according to the procedure described below.

The strain was cultured on YM agar for 48 h at 30 °C. The colonies were transferred to plastic tubes, the cells were washed with 1 mL of ultrapure water and centrifuged at 70,000× *g* for 2 min, and the supernatant was discarded. This process was repeated once. Subsequently, 1 mL of 75% ethanol (*v*/*v*) was added to the precipitate, which was incubated at room temperature for 10 min and then centrifuged. The supernatant was discarded, and the precipitate was dried in an oven at 36 ± 1 °C, then resuspended in a solution of 70% formic acid and acetonitrile (1:1). The extracts obtained were centrifuged at 70,000× *g* for 2 min, and 1 µL of the supernatants was deposited onto a plate for mass spectrometer analysis, being air-dried at room temperature. Then, 1 µL of the matrix α-cyano-4-hydroxycinnamic acid (10 mg/mL) dissolved in acetonitrile/2.5% trifluoroacetic acid (1:1) was added to the sample and air-dried (Sauer, Freiwald et al. 2008).

The sample was inoculated in triplicate on a stainless steel plate, which was subsequently introduced into a Microflex LT MALDI-TOF MS mass spectrometer equipped with a 337 nm nitrogen laser in linear mode, controlled by FlexControl 3.3 software (Bruker Daltonics, Billerica, MA USA). Mass spectra were collected in the mass range of 2000 to 20,000 *m*/*z*.

The spectra obtained were analyzed using MALDI Biotyper software version 2.0 (Bruker Daltonics, Billerica, MA, USA) with standard settings for identification. The algorithm used by the MALDI Biotyper compares the spectra of the unknown sample with reference samples contained in the database. The procedure takes into account the masses and relative intensities of the unknown spectra [22]. As specified by the manufacturer, the criteria for identification are: scores ≥ 2 for reliable species identification and between 1.7 and 1.9 for reliable genus identification. Identification scores below 1.7 are considered unreliable, making identification impossible. As a control for protein extraction and reading, the *Escherichia coli* strain was used.

2.5. Fermentation of the Selected Strain with Agro-Industrial Residues

Fermentations were carried out in a medium containing residues of passion fruit, pineapple, and guava. For the fermentation, a pre-inoculum composed of YM medium and three loops of the selected strain was prepared. This pre-inoculum was incubated in a shaker at 30 °C for 48 h, with a rotation of 150 rpm. After the pre-inoculum incubation period, complete fermentations and chemical and biological blanks were prepared for each batch of fermentation performed with each residue, as follows: biological blank, containing the same amount of culture medium and inoculum; chemical blank, containing the same amount of culture medium and residue; and complete fermentation, containing the same amount of culture medium, inoculum, and residue. The fermentations were conducted in amber flasks, which were incubated in a shaker at 25 °C for five days, with a rotation of 75 rpm.

2.6. Analysis of Volatile Compounds

During the fermentation incubation period, the amber flasks were removed daily, corresponding to the times of 0 h, 24 h, 48 h, 72 h, and 96 h, for the biological and chemical blanks, as well as for the complete fermented product. Thus, three flasks were removed per day. The volatile compounds were separated using the SPME technique with a DVB/CAR/PDMS fiber (50/30 µm divinylbenzene/carboxen/polydimethylsiloxane; Supelco, Bellefonte, PA, USA) and injected into a gas chromatograph (AGILENT, model

7980C) coupled to a mass spectrometer (MS), with an ionization voltage of 70 eV. The compounds were separated in a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm).

The conditions used in the GC-MS system were as follows: initial oven temperature of 30 °C for 5 min, increasing by 3 °C per minute until reaching 240 °C, and maintaining at this temperature for 10 min. The injector temperature was set at 220 °C. Helium was used as the carrier gas, with a flow rate of 1.0 mL per minute in the splitless injection system. The transfer line temperature was 170 °C, and the mass scan range was from 35 to 350 u.m.a. The compounds were identified by comparing their mass spectra with the NIST (National Institute of Standards & Technology, Gaithersburg, MD, USA) database and by linear retention indices, calculated based on the retention times of a series of n-alkanes, analyzed under the same separation conditions.

2.7. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and Tukey's test, with a significance level of 5%, using Statistical Analysis System (SAS) software.

3. Results and Discussion

3.1. Isolation and Selection of Aroma-Producing Microorganisms

The application of the isolation technique revealed the presence of 37 microorganisms in the selected fruits. Of these, 24 were yeasts and 13 were fungi. The presence of endophytic microorganisms was found in all the fruits used for isolation, as detailed in Table 1. After isolation, the yeasts were cultured in liquid medium with 5% fructose and 0.5% yeast extract in a shaker at 30 °C and 150 rpm for 72 h.

Table 1. Number of microorganisms isolated from each fruit.

Fruits	Number of Yeasts Isolated	Number of Fungi Isolated
Strawberry	10	3
Lychee	8	2
Tangerine	6	8

The fermented products with yeast strains isolated from strawberry showed the highest ratings in aroma intensity. Among the evaluated attributes, the fruity aroma received the highest rating, as observed in Figure 1. The analysts also identified that the HIM-01 strain had an aroma similar to that of strawberry. Studies indicate that yeasts isolated from fruits, such as strawberries, produce a variety of volatile compounds that significantly contribute to the aromatic profile of fermented products. Yeasts like *Saccharomyces cerevisiae* are known to produce higher levels of alcohols, esters, aldehydes, and terpenes, which are responsible for fruity and floral aromas in wines and ciders [23,24].

The fermented products with endophytic yeasts isolated from lychee, as presented in Figure 2, exhibited fermented, alcoholic, and sweet aromas, with the LIC-01 strain receiving the highest ratings in these attributes. Yeasts produce a diversity of volatile compounds during fermentation, including esters and alcohols, which are known for their contributions to fermented and sweet aromas [25].

For the fermentations with yeasts isolated from tangerine, presented in Figure 3, the highest intensity ratings were observed for the odors "sweet", "fruity", and "citrus" in strains TAM-01, TAM-02, and TAM-03, respectively. An aroma attribute not previously reported by the analysts and classified as "herbal" was detected only in strains TAM-03, TAM-04, and TAM-05, with the highest average rating (3) observed in strain TAM-05. The diversity of volatile compounds produced by these yeasts can explain the variety of observed aromas, including esters and alcohols contributing to citrus and herbal aromas [24,25].

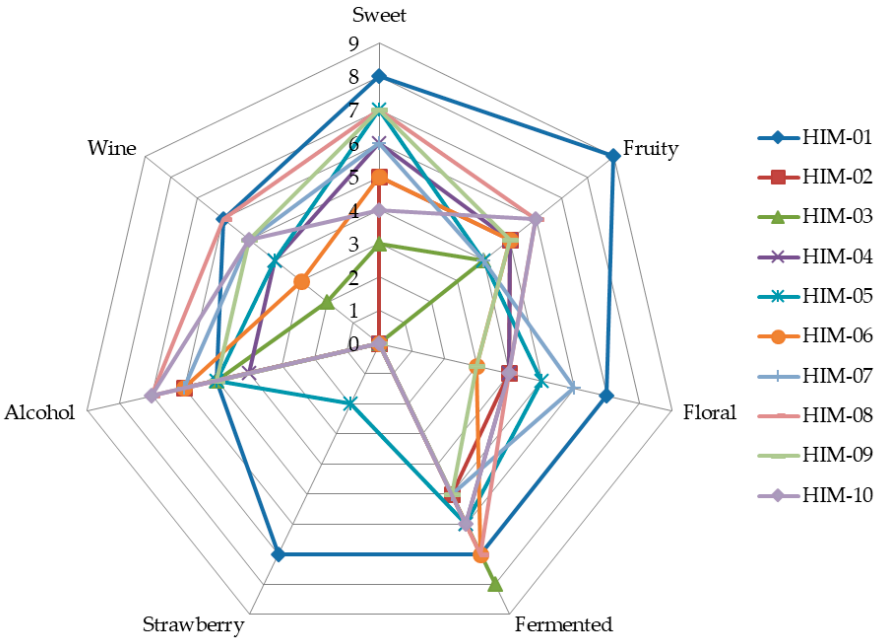


Figure 1. Ratings of various aromas observed in fermented products obtained using microorganisms isolated from strawberry in synthetic medium.

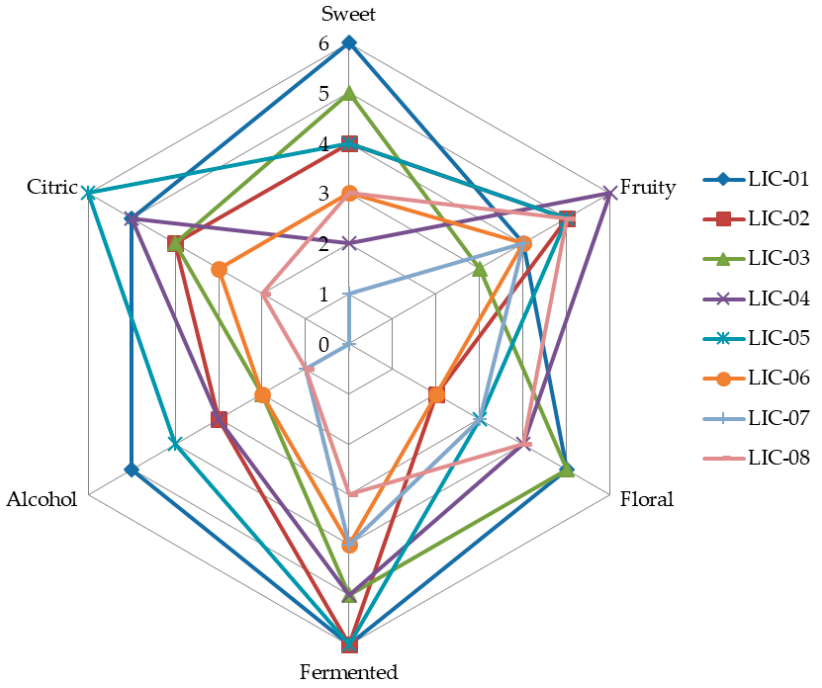


Figure 2. Ratings of various aromas observed in fermented products obtained using microorganisms isolated from lychee in synthetic medium.

In general, the panelists reported 11 sensory attributes, most of which had pleasant aroma characteristics, such as sweet, fruity, floral, and citrus notes. The remaining characteristic odors were non-significant aromatic notes, such as fermented and alcoholic. The best aroma ratings and attributes were for the HIM-01 strain, isolated from strawberry, which also received the strawberry aroma rating in its sensory analysis. Due to its potential for producing fruity and sweet aromas, the HIM-01 strain was chosen to continue the fermentation experiments in media containing agro-industrial residues.

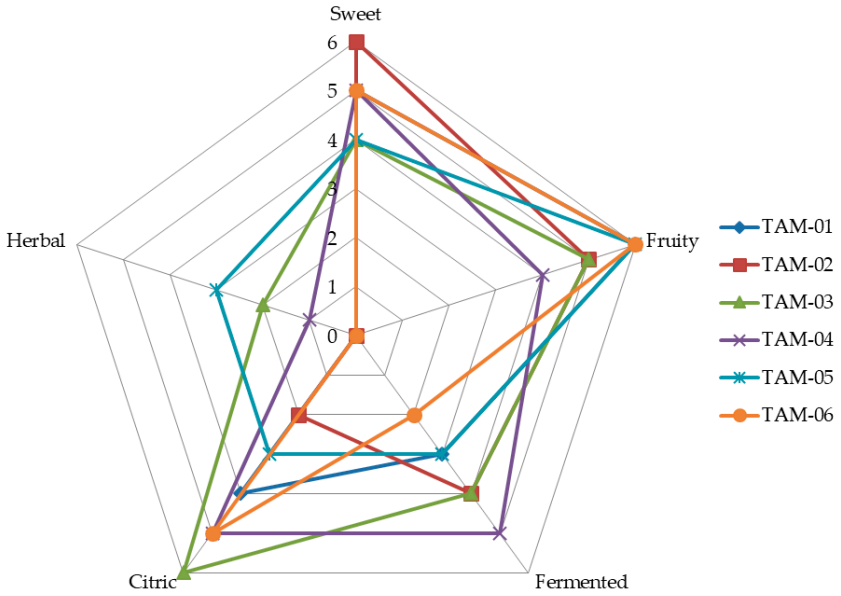


Figure 3. Ratings of various aromas observed in fermented products obtained using microorganisms isolated from tangerine in synthetic medium.

3.2. Identification of the HIM-01 Strain by MALDI-TOF-MS

The results of the identification of the HIM-01 strain by microbiological culture using MALDI-TOF/MS are presented in Table 2. The mass spectrum provided by MALDI-TOF/MS for the yeast *Kloeckera apiculata* is shown in Figure 4. This allows for demonstration of the ribosomal protein profile of this yeast. The methodology of taxonomic identification by MALDI-TOF/MS is based on the phenotypic characteristics of microorganisms, using a specific mass generated in the spectrum by bacterial proteins, and subsequent comparison of the mass spectrum. Thus, the quality of the spectrum is evaluated considering factors such as the number of peaks, signal-to-noise ratio (S), resolution, and signal intensity [26–28].

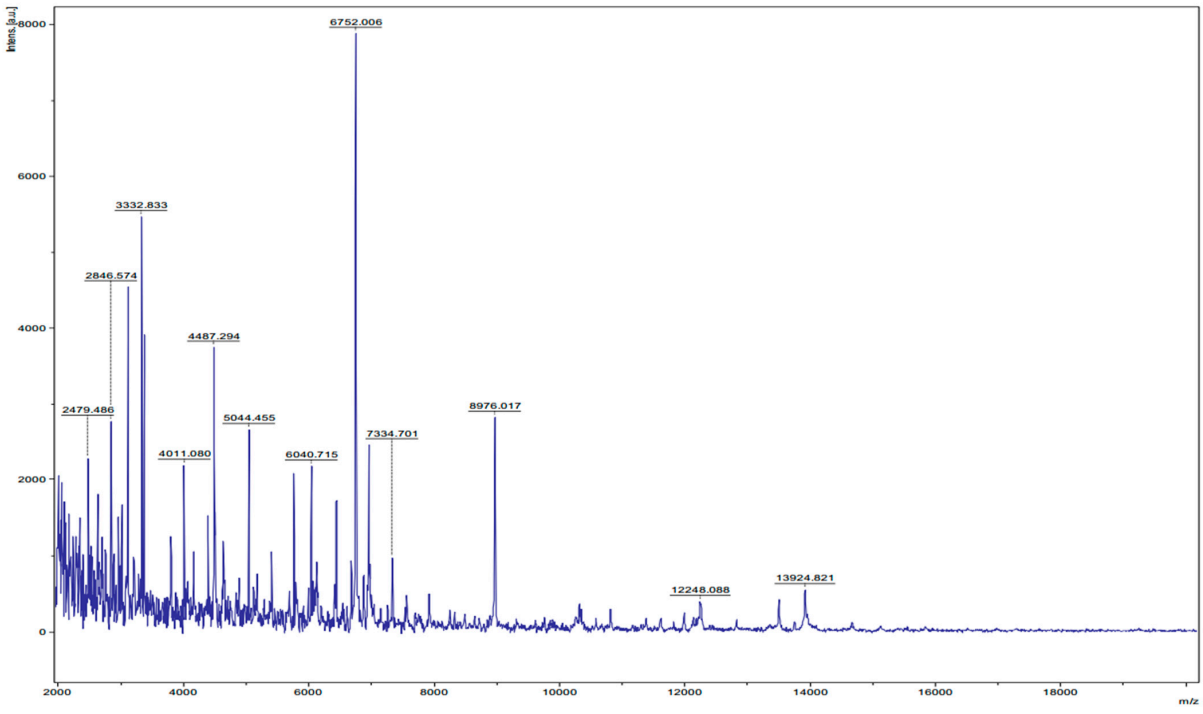


Figure 4. MALDI-TOF/MS spectrum of *Kloeckera apiculata*.

Table 2. Volatile compounds identified in the fermented product after 72 h using passion fruit residue and the *Kloeckera apiculata* strain.

N°	Compounds	IRCal	Area (%)	B.Q. *	B.B. *	Aroma [b]
1	Ethanol	522	10.03		x	alcoholic
2	Methyl acetate	560	10.72		x	sweet, fruity
3	2-Methyl-1-propanol	550	1.81	x		
4	Ethyl acetate	640	11.14		x	fruity
5	Isobutyl acetate	790	0.67		x	fruity
6	Hexanal	800	0.47		x	green
7	Ethyl butanoate	802	1.74		x	fruity
8	(*Z*)-3-Hexen-1-ol	855	1.12	x		
9	1-hexanol	863	6.07			herbs
10	isoamyl acetate	877	5.95	x		fruity
11	2-Methylbutyl acetate	879	1.35	x		
12	Styrene	890	0.63	x		
13	2-Heptanol	900	0.60		x	citrus
14	Methyl hexanoate	925	0.56		x	fruity
15	*α*-Thujene	934	0.54	x		
16	Benzaldehyde	960	6.33	x		
17	*β*-Myrcene	991	3.02			spicy
18	*β*-Cymene	1019	1.12	x		
19	(*E*)-Ocimene	1029	0.84	x		herbs
20	Benzeneacetaldehyde	1035	3.30	x		green
21	3-Carene	1038	4.47			sweet
22	4-(benzoylmethyl)-6-methyl-2H-1,4-benzoxazin-3-one	1058	0.56	x		
23	Terpinolene	1076	0.98		x	herbs
24	Methyl benzoate	1082	6.51		x	phenolic
25	2-Phenylethyl acetate	1104	14.54			roses
26	Ethyl benzoate	1165	2.68	x		mint
27	Hexyl 2-methylpropanoate	1189	2.26			

B.Q. *: Chemical blank; B.B. *: biological blank; x: indicates the presence of the compound in the blanks; [b]: Goodner, 2008.

The microorganism identified as *Kloeckera apiculata* is a yeast commonly found in the early stages of the alcoholic fermentation process, especially in wines. These yeasts are known as "non-*Saccharomyces* yeasts" and have been extensively studied due to the formation of metabolites that directly affect the final aroma of fermented products [29–31]. Yeasts of the genus *Kloeckera* have the potential to produce various aromatic compounds of interest due to their ability to biosynthesize and/or release these compounds in cultures under appropriate working conditions.

3.3. Volatile Compounds Obtained from the Fermentation of Kloeckera Apiculata in Culture Medium Supplemented with Agro-Industrial Residues

After identifying *K. apiculata* as the microorganism with the best performance in sensory analysis, fermentations were carried out in medium containing agro-industrial residues of passion fruit, guava, and pineapple. These fermentations were performed in triplicate, and chromatographic analyses were conducted daily at 0, 24, 48, 72, and 96 h. The optimal time for all fermentation processes was 72 h. Simultaneously, experiments with biological blanks and chemical blanks were conducted.

Table 2 presents all the compounds identified in the product obtained by the fermentation of *Kloeckera apiculata* with passion fruit residue. Among the identified compounds, esters stand out as the major class. Esters are compounds responsible for fruity and floral aroma notes [32,33], with the main ones being 2-phenylethyl acetate (14.54%), ethyl acetate (11.14%), methyl acetate (10.72%), and methyl benzoate (6.51%). Studies reveal that esters are mostly obtained through low levels of 2-phenylethanol (together with acetyl-CoA), which synthesize 2-phenylethyl acetate in conjunction with acetyltransferase. Yeasts con-

vert alcohols into their corresponding acetates with high yield (over 90% for 3-methyl-butyl acetate) [34,35]. Liu et al. [36] revealed that *K. apiculata* strains have sufficient quantities of 2-phenylethanol in their cell walls for extraction and use as an antimicrobial; this fact may explain the production of 2-phenylethyl acetate in the medium with the residue. However, it is necessary to consider that the chemical blank analyses also found the presence of 2-phenylethanol, which may have been used as a source for the production of the esters found. Another study demonstrated that the addition of l-phenylalanine during co-fermentation significantly increased the production of 2-phenylethyl acetate [37]. Additionally, research has shown that the production of β-phenylethyl acetate by non-*Saccharomyces* yeasts contributes to complex and desirable aromatic notes, with this ester being one of the main ones found in alcoholic fermentations using *Kloeckera* strains [38,39].

Another way of producing esters, especially ethyl acetate by *K. apiculata*, comes from the reaction of saturated fatty acids with alcohols formed during alcoholic fermentation. Ethyl acetate is the most abundant ester found in alcoholic fermentations using *Kloeckera* strains [29,40,41]. Since passion fruit residue has high amount of lipids, the yeast is possibly using the fatty acids from the medium to produce ethyl acetate, explaining its high concentration.

Alcohols originate from the metabolism of amino acids or sugars during alcoholic fermentation carried out by yeasts [42]. They are divided into aliphatic alcohols and aromatic alcohols. Aliphatic alcohols include compounds such as propanol, isoamyl alcohol, and isobutanol. Aromatic alcohols consist of compounds such as 2-phenylethanol and tyrosol. The concentration of these compounds in fermentation as a final product can be influenced by the concentration of amino acids in the culture medium, as well as by various parameters such as the presence of ethanol, temperature, pH, agitation, and soluble solids [43]. In the fermentation with passion fruit residues, the alcohols identified in the highest percentages were ethanol (10.03%) and 1-hexanol (6.07%). The production of ethanol is an indicator of alcoholic fermentation, and hexanol is an important primary alcohol, widely used in the cosmetics and fragrance industry as a fixative. These two compounds were also identified in the biological blanks on all fermentation days.

Other classes of compounds were identified in smaller quantities, such as terpenes and aldehydes. The identified terpenes were 3-carene (4.47%), β-myrcene (3.02%), β-cymene (1.12%), terpinolene (0.98%), and (*E*)-ocimene (0.84%). The formation of terpenic compounds in fermentation media using yeasts such as *K. apiculata* often occurs through the hydrolysis of glycosides caused by the action of enzymes produced by this microorganism, such as β-glucosidase. However, the production of terpenes can be limited by the concentration of ethanol [44]. In the present work, ethanol was one of the alcohols detected in the highest concentrations, which may have influenced the inhibition of terpene formation. Among the identified terpenes, 3-carene was the only compound not found in either of the blanks (chemical and biological). For aldehydes, only two compounds were identified in the fermented product with passion fruit residue: benzeneacetaldehyde (3.30%) and hexanal (0.47%), both also found in the chemical blanks, being pre-existing compounds in the residue.

The guava residue used as a substrate resulted in the identification of 22 volatile compounds in the fermented product, as presented in Table 3. The major compounds identified were the terpenes aromadendrene (41.23%), humulene (7.06%), α-selinene (6.48%), δ-cadinene (6.29%), and β-caryophyllene (6.26%). The chemical control also revealed the presence of these terpenic compounds. However, the presence of these compounds showed interesting behavior. The area of β-caryophyllene was reduced from 47.67% to 6.10% in the chemical control. The areas of α-selinene and δ-cadinene also decreased, from 7.44% and 7.26% to 6.31% and 6.13%, respectively. On the other hand, the area of aromadendrene increased from 8% to 40.17%, and humulene decreased from 8.25% to 6.88%. The reduction in β-caryophyllene concentration may be related to its degradation by oxygen during the fermentation period. These terpenic compounds were also identified in studies on guava pulp by Nunes et al. [45]. Analyzing the volatile composition of fresh and freeze-dried

guava pulp, the authors found 16 terpenic compounds, all of which were present in this study, both in the fermented product with guava residue and in the chemical control.

Table 3. Volatile compounds identified in the fermented product after 72 h using guava residue and *Kloeckera apiculata* strain.

N°	Compounds	IRCal	Area (%)	B.Q. *	B.B. *	Aroma [b]
1	Ethanol	529	2.96	x	x	alcohol
2	Ethyl acetate	644	7.89		x	fruity
3	Isoamyl alcohol	775	1.26	x		
4	2-Methyl-1-butanol	777	0.78	x		
5	Isoamyl acetate	877	4.34		x	
6	*D*-limonene	1022	1.21	x		citric
7	Eucalyptol	1024	0.53	x		eucalyptus
8	(*E*)-Ocimene	1029	3.21		x	
9	2-Phenylethanol	1038	0.57		x	floral
10	α-Pyronene	1109	0.55			
11	2-Phenylethyl acetate	1117	0.42	x		floral, roses
12	α-Copaene	1259	0.87	x		woody
13	β-Caryophyllen	1374	6.26		x	sweet
14	Aromandendrene	1419	41.23		x	woody
15	Humulene	1437	7.06	x		woody
16	Alloaromadendrene	1451	4.55	xx		woody
17	γ-Muurolene	1458	0.85	x		herbal
18	β-Selinene	1474	1.22	x		herbal
19	α-Selinene	1483	6.48	x		Waxy
20	δ-Cadinene	1492	6.29	x		herbal
21	(+)-Ledene	1521	0.87	x		
22	β-Guaiene	1579	0.57	x		balsamic

B.Q. *: Chemical blank; B.B. *: biological blank; x: indicates the presence of the compound in the blanks; [b]: Goodner, 2008.

Another important aromatic compound identified was ethyl acetate (7.89%). The presence of this ester contributes to the formation of aroma notes with fruity and sweet characteristics. Ethyl acetate is formed during fermentation by the reaction of alcohols and acetyl-CoA, catalyzed by the enzyme alcohol acetyltransferase. Ethanol is the main alcohol in fermentations with *K. apiculata*, and therefore ethyl acetate, originating from ethanol and acetyl-CoA, is the most abundant ester in fermentation processes with this microorganism. Zohre and Erten [46] also found high production of ethyl acetate in fermentation processes with *K. apiculata*. The decrease in the ethanol area in the biological control, from 15.08% to 2.88% in the fermented product, indicates that it was consumed in the reaction forming ethyl acetate.

Fermentation of *K. apiculata* with guava residue did not result in the formation of new compounds through biotransformation. However, the residue proved to be promising for the production of esters such as ethyl acetate, likely due to its composition that facilitates the production of ethanol, which can be used in reactions to obtain these esters.

Pineapple residue was used as a substrate for fermentation with *Kloeckera apiculata*. A total of 41 compounds were identified in the fermentations with this substrate, as shown in Table 4. The compound found in the highest concentration was furfural (37.09%), a product of the hydrolysis of sugars present in the hemicellulose of plants. Pardo et al. [47] showed that the concentration of hemicellulose can reach 28.69 g/100 g in pineapple residues, making this residue an important source for obtaining furfural. Biotechnologically, furfural can be obtained from the degradation of xylose under acidic conditions, reaching high concentrations depending on the substrate used. During this hydrolysis process, inhibitory compounds for microbial development, such as acetic acid, sugar derivatives, and lignin degradation products, are formed and released into the medium [48,49]. These inhibitory compounds can reduce or even render ethanol production unfeasible in alcoholic

fermentations [50,51]. In the present study, the high concentration of furfural may have influenced the absence of ethanol among the identified compounds, considering that this alcohol was produced in the fermentations with passion fruit and guava residues. Another compound related to furfural is furfuryl alcohol, resulting from its degradation, found at a concentration of 0.64% in the fermented products.

Table 4. Volatile compounds identified in fermented products after 72 h using pineapple residue and the *Kloeckera apiculata* strain.

N°	Compounds	IRCal	Area (%)	B.Q. *	B.B. *	Aroma [b]
1	Isoamyl alcohol	775	4.72		x	fruity
2	2-Methyl-1-butanol	776	5.10		x	burned
3	(*E*)-2-Methylcyclopentanol	800	0.46	x		
4	2,4-Dimethylheptane	821	0.89		x	
5	Furfural	832	37.09		x	
6	2-Ethoxy-2-cyclohexenone	841	9.97	x		
7	1,3-Dimethylheptane	863	1.67	x		
8	Isoamyl acetate	877	18.45		x	fruity
9	2-Methylbutyl acetate	880	2.49		x	fruity
10	Methional	906	0.45		x	
11	2-Acetylfuran	913	0.42		x	
12	Benzaldehyde	961	1.89		x	
13	2-Pentylfuran	991	0.91		x	
14	2-Furanmethanol	995	0.80		x	
15	Ethyl hexanoate	999	1.60			fruity
16	4-Methyldecane	1007	0.99			
17	3-(prop-2-enoyloxy)tetradecane	1017	0.75	x		
18	Benzeneacetaldehyde	1035	3.46	x		green
19	1-Propyldecyl phenylacetate	1040	0.71		x	
20	1-Methylodecyl phenylacetate	1043	0.56		x	
21	2,6,6-trimethyl-bicyclo[3.1.1]hept-3-ylamine	1091	0.37	x		
22	Ethyl octanoate	1195	3.90			wax
23	2-Phenylethyl acetate	1261	2.36			

B.Q. *: Chemical blank; B.B *: biological blank; x: indicates the presence of the compound in the blanks; [b]: Goodner, 2008.

The second most abundant compound was isoamyl acetate (18.45%). However, this compound was also detected in small concentrations in the control, as pineapple residues have a high concentration of esters in their volatile composition. Esters such as 2-phenylethyl acetate (2.36%) and 2-methylbutyl acetate (2.49%) were also found. Mamede and Pastore [30], analyzing volatile compounds in *K. apiculata* grape fermentation musts, reported the presence of ethyl propionate and propyl acetate in higher concentrations. Temperature is a decisive factor in the concentration and formation of esters such as ethyl acetate, ethyl butyrate, isoamyl acetate, and ethyl hexanoate in fermentation processes using yeasts like *Kloeckera apiculata* [52]. Other compounds were also detected, such as isoamyl alcohol (4.72%) and 2-methyl-1-butanol (5.10%), and aldehydes like benzaldehyde (1.89%) and benzeneacetaldehyde (3.46%). These compounds were found in the control, not resulting from biotransformation caused by the interaction of the microorganism with the medium.

4. Conclusions

In total, 37 endophytic microorganisms were isolated, of which 24 were yeasts and 13 were endophytic fungi. Among these, the yeast strain HIM-01, isolated from strawberry, showed the best sensory results, with sweet and fruity aroma notes. The HIM-01 strain was identified as the microorganism *Kloeckera apiculata* using the MALDI-TOF/MS technique in a rapid, simple, and unequivocal manner. In the fermented products, 27, 23, and 22 compounds were identified from passion fruit, pineapple, and guava residues, respec-

tively. Considering the number and diversity of compounds found in the fermented products derived from passion fruit residues, it can be concluded that this residue is a promising substrate for obtaining aromatic compounds. Notable compounds included ethyl acetate (11.14%) and 2-phenylethyl acetate (14.54%), which contributed to fruity and floral aromas, respectively. This study demonstrates that the use of agro-industrial residues can be an effective and sustainable strategy for the production of high-value compounds, leveraging endophytic microorganisms isolated from tropical fruits.

Author Contributions: Conceptualization, M.T.S.L.N.; methodology, M.T.S.L.N. and R.A.R.D.S.; software, M.T.S.L.N. and R.D.D.S.; formal analysis, M.T.S.L.N. and R.A.R.D.S.; investigation, M.T.S.L.N.; resources, N.N.; data curation, M.T.S.L.N., R.A.R.D.S., M.S.D.J., H.C.S.A. and R.D.D.S.; writing—original draft preparation, M.T.S.L.N., L.C.L.D.A.S. and N.N.; writing—review and editing, L.C.L.D.A.S. and N.N.; visualization, N.N.; supervision, N.N.; project administration, N.N.; funding acquisition, N.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil. The research project Instituto Nacional de Ciência e Tecnologia de Frutos Tropicais (Project 465335/2014-4) helped in developing this work, and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Financial code 001), Brazil, provided fellowships.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sandes, R.D.D.; dos Santos, R.A.R.; de Jesus, M.S.; Araujo, H.C.S.; Leite Neta, M.T.S.; Rajkumar, G.; Narain, N. Agro-Industrial Residues Used as Substrates for the Production of Bioaroma Compounds with Basidiomycetes: A Comprehensive Review. *Fermentation* **2024**, *10*, 23. [CrossRef]
2. Bicas, J.L.; Silva, J.C.; Dionísio, A.P.; Pastore, G.M. Biotechnological production of bioflavors and functional sugars. *Food Sci. Technol.* **2010**, *30*, 7–18. [CrossRef]
3. Krings, U.; Berger, R.G. Biotechnological production of flavours and fragrances. *Appl. Microbiol. Biotechnol.* **1998**, *49*, 1–8. [CrossRef]
4. Maróstica Júnior, M.R.; Pastore, G.M. Biotransformação de limoneno: Uma revisão das principais rotas metabólicas. *Quim. Nova* **2007**, *30*, 382–387. [CrossRef]
5. Medeiros, A.B.; Pandey, A.; Vandenberghe, L.P.; Pastore, G.M.; Soccol, C.R. Production and Recovery of Aroma Compounds Produced by Solid-State Fermentation Using Different Adsorbents. *Food Technol. Biotechnol.* **2006**, *44*, 47–51.
6. Uenojo, M.; Pastore, G.M. β-carotene biotransformation to obtain aroma compounds. *Food Sci. Technol.* **2010**, *30*, 822–827. [CrossRef]
7. Aggelopoulos, T.; Katsieris, K.; Bekatorou, A.; Pandey, A.; Banat, I.M.; Koutinas, A.A. Solid state fermentation of food waste mixtures for single cell protein, aroma volatiles and fat production. *Food Chem.* **2014**, *145*, 710–716. [CrossRef]
8. Saikkonen, K. Forest structure and fungal endophytes. *Fungal Biol. Rev.* **2007**, *21*, 67–74. [CrossRef]
9. Sun, L.; Qiu, F.; Zhang, X.; Dai, X.; Dong, X.; Song, W. Endophytic Bacterial Diversity in Rice (*Oryza sativa* L.) Roots Estimated by 16S rDNA Sequence Analysis. *Microb. Ecol.* **2008**, *55*, 415–424. [CrossRef]
10. Qin, S.; Xing, K.; Jiang, J.-H.; Xu, L.-H.; Li, W.-J. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 457–473. [CrossRef]
11. Thomas, P.; Kumari, S.; Swarna, G.K.; Prakash, D.P.; Dinesh, M.R. Ubiquitous presence of fastidious endophytic bacteria in field shoots and index-negative apparently clean shoot-tip cultures of papaya. *Plant Cell Rep.* **2007**, *26*, 1491–1499. [CrossRef] [PubMed]
12. Lo Piccolo, S.; Ferraro, V.; Alfonzo, A.; Settanni, L.; Ercolini, D.; Burruano, S.; Moschetti, G. Presence of endophytic bacteria in *Vitis vinifera* leaves as detected by fluorescence in situ hybridization. *Ann. Microbiol.* **2010**, *60*, 161–167. [CrossRef]
13. Melnick, R.L.; Suárez, C.; Bailey, B.A.; Backman, P.A. Isolation of endophytic endospore-forming bacteria from *Theobroma cacao* as potential biological control agents of cacao diseases. *Biol. Control* **2011**, *57*, 236–245. [CrossRef]
14. Guo, B.; Wang, Y.; Sun, X.; Tang, K. Bioactive natural products from endophytes: A review. *Appl. Biochem. Microbiol.* **2008**, *44*, 136–142. [CrossRef]
15. Hardoim, P.R.; van Overbeek, L.S.; van Elsas, J.D. Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol.* **2008**, *16*, 463–471. [CrossRef] [PubMed]
16. Rodrigues, K.F.; Samuels, G.J. Fungal endophytes of *Spondias mombin* leaves in Brazil. *J. Basic Microbiol.* **1999**, *39*, 131–135. [CrossRef]

17. Pereira, J.O.; Vieira, M.L.C.; Azevedo, J.L. Endophytic fungi from *Musa acuminata* and their reintroduction into axenic plants. *World J. Microbiol. Biotechnol.* **1999**, *15*, 37–40. [CrossRef]
18. Uenojo, M.; Pastore, G.M. Isolamento e seleção de microrganismos pectinolíticos a partir de resíduos provenientes de agroindústrias para produção de aromas frutais. *Food Sci. Technol.* **2006**, *26*, 509–515. [CrossRef]
19. Soccol, C.R.; Vandenberghe, L.P.S. Overview of applied solid-state fermentation in Brazil. *Biochem. Eng. J.* **2003**, *13*, 205–218. [CrossRef]
20. Medeiros, A.B.; Pandey, A.; Freitas, R.J.; Christen, P.; Soccol, C.R. Optimization of the production of aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation using factorial design and response surface methodology. *Biochem. Eng. J.* **2000**, *6*, 33–39. [CrossRef]
21. Pandey, A.; Soccol, C.R.; Nigam, P.; Soccol, V.T. Biotechnological potential of agro-industrial residues. I: Sugarcane bagasse. *Bioresour. Technol.* **2000**, *74*, 69–80. [CrossRef]
22. Lartigue, M.-F.; Héry-Arnaud, G.; Haguenoer, E.; Domelier, A.-S.; Schmit, P.-O.; Van Der Mee-Marquet, N.; Lanotte, P.; Mereghetti, L.; Kostrzewa, M.; Quentin, R. Identification of *Streptococcus agalactiae* isolates from various phylogenetic lineages by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J. Clin. Microbiol.* **2009**, *47*, 2284–2287. [CrossRef] [PubMed]
23. Tekarslan-Sahin, S.H. Adaptive Laboratory Evolution of Yeasts for Aroma Compound Production. *Fermentation* **2022**, *8*, 372. [CrossRef]
24. Aslankoohi, E.; Herrera-Malaver, B.; Rezaei, M.N.; Steensels, J.; Courtin, C.M.; Verstrepen, K.J. Non-Conventional Yeast Strains Increase the Aroma Complexity of Bread. *PLoS ONE* **2016**, *11*, e0165126. [CrossRef]
25. Chen, L.; Li, K.; Chen, H.; Li, Z. Reviewing the Source, Physiological Characteristics, and Aroma Production Mechanisms of Aroma-Producing Yeasts. *Foods* **2023**, *12*, 3501. [CrossRef]
26. Seng, P.; Drancourt, M.; Gouriet, F.; La Scola, B.; Fournier, P.-E.; Rolain, J.M.; Raoult, D. Ongoing Revolution in Bacteriology: Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry. *Clin. Infect. Dis.* **2009**, *49*, 543–551. [CrossRef]
27. Drevinek, M.; Dresler, J.; Klimentova, J.; Pisa, L.; Hubalek, M. Evaluation of sample preparation methods for MALDI-TOF MS identification of highly dangerous bacteria. *Lett. Appl. Microbiol.* **2012**, *55*, 40–46. [CrossRef] [PubMed]
28. Schumaker, S.; Borror, C.M.; Sandrin, T.R. Automating data acquisition affects mass spectrum quality and reproducibility during bacterial profiling using an intact cell sample preparation method with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 243–253. [CrossRef]
29. Mamede, M.E.; Cardello, H.M.; Pastore, G.M. Evaluation of an aroma similar to that of sparkling wine: Sensory and gas chromatography analyses of fermented grape musts. *Food Chem.* **2005**, *89*, 63–68. [CrossRef]
30. Mamede, M.E.; Pastore, G.M. Study of methods for the extraction of volatile compounds from fermented grape must. *Food Chem.* **2006**, *96*, 586–590. [CrossRef]
31. Ciani, M.; Beco, L.; Comitini, F. Fermentation behaviour and metabolic interactions of multistarter wine yeast fermentations. *Int. J. Food Microbiol.* **2006**, *108*, 239–245. [CrossRef]
32. Sandes, R.D.D.; De Jesus, M.S.; Araujo, H.C.S.; Dos Santos, R.A.R.; Nogueira, J.P.; Leite Neta, M.T.S.; Narain, N. The Production of Bioaroma by *Auriporia aurulenta* Using Agroindustrial Waste as a Substrate in Submerged Cultures. *Fermentation* **2023**, *9*, 593. [CrossRef]
33. Lee, P.R.; Ong, Y.L.; Yu, B.; Curran, P.; Liu, S.Q. Profile of volatile compounds during papaya juice fermentation by a mixed culture of *Saccharomyces cerevisiae* and *Williopsis saturnus*. *Food Microbiol.* **2010**, *27*, 853–861. [CrossRef] [PubMed]
34. Gatfield, I.L. Production of flavor and aroma compounds by biotechnology. *Food Technol.* **1988**, *42*, 110–122.
35. Welsh, F. Overview of bioprocess flavor and fragrance production. In *Bioprocess Production of Flavor, Fragrance, and Color Ingredients*; Wiley & Sons Ltd.: Hoboken, NJ, USA, 1994; pp. 1–17.
36. Liu, P.; Cheng, Y.; Yang, M.; Liu, Y.; Chen, K.; Long, C.-a.; Deng, X. Mechanisms of action for 2-phenylethanol isolated from *Kloeckera apiculata* in control of *Penicillium molds* of citrus fruits. *BMC Microbiol.* **2014**, *14*, 242. [CrossRef]
37. Adame-Soto, P.J.; Aréchiga-Carvajal, E.T.; López, M.G.; González-Herrera, S.M.; Moreno-Jiménez, M.R.; Urtiz-Estrada, N.; Rutiaga-Quiñones, O.M. Potential production of 2-phenylethanol and 2-phenylethylacetate by non-*Saccharomyces yeasts* from *Agave durangensis*. *Ann. Microbiol.* **2019**, *69*, 989–1000. [CrossRef]
38. Martin, V.; Valera, M.J.; Medina, K.; Boido, E.; Carrau, F. Oenological Impact of the *Hanseniaspora/Kloeckera* Yeast Genus on Wines—A Review. *Fermentation* **2018**, *4*, 76. [CrossRef]
39. de Lima, L.A.; Diniz, R.H.S.; de Queiroz, M.V.; Fietto, L.G.; da Silveira, W.B. Screening of Yeasts Isolated from Brazilian Environments for the 2-Phenylethanol (2-PE) Production. *Biotechnol. Bioprocess Eng.* **2018**, *23*, 326–332. [CrossRef]
40. Romano, P.; Palla, G.; Caligiani, A.; Brandolini, V.; Maietti, A.; Salzano, G. Evaluation of stereoisomers of 2,3-butanediol and acetoin to differentiate *Saccharomyces cerevisiae* and *Kloeckera apiculata* wine strains. *Biotechnol. Lett.* **2000**, *22*, 1947–1951. [CrossRef]
41. Bilbao, A.; Irastorza, A.; Dueñas, M.; Fernandez, K. The effect of temperature on the growth of strains of *Kloeckera apiculata* and *Saccharomyces cerevisiae* in apple juice fermentation. *Lett. Appl. Microbiol.* **1997**, *24*, 37–39. [CrossRef]
42. Zhang, L.; Shen, Y.; Wang, F.; Leng, Y.; Liu, J.-K. Rare merosesquiterpenoids from basidiomycete *Craterellus odoratus* and their inhibition of 11β-hydroxysteroid dehydrogenases. *Phytochemistry* **2010**, *71*, 100–103. [CrossRef]
43. Hernández-Orte, P.; Cacho, J.F.; Ferreira, V. Relationship between Varietal Amino Acid Profile of Grapes and Wine Aromatic Composition. Experiments with Model Solutions and Chemometric Study. *J. Agric. Food Chem.* **2002**, *50*, 2891–2899. [CrossRef]

44. Gunata, Y.Z.; Bayonove, C.L.; Tapiero, C.; Cordonnier, R.E. Hydrolysis of grape monoterpenyl. beta.-D-glucosides by various. beta.-glucosidases. *J. Agric. Food Chem.* **1990**, *38*, 1232–1236. [CrossRef]
45. Nunes, J.C.; Lago, M.G.; Castelo-Branco, V.N.; Oliveira, F.R.; Torres, A.G.; Perrone, D.; Monteiro, M. Effect of drying method on volatile compounds, phenolic profile and antioxidant capacity of guava powders. *Food Chem.* **2016**, *197*, 881–890. [CrossRef]
46. Zohre, D.E.; Erten, H. The influence of Kloeckera apiculata and Candida pulcherrima yeasts on wine fermentation. *Process Biochem.* **2002**, *38*, 319–324. [CrossRef]
47. Pardo, M.E.S.; Cassellis, M.E.R.; Escobedo, R.M.; García, E.J. Chemical characterisation of the industrial residues of the pineapple (*Ananas comosus*). *J. Agric. Chem. Environ.* **2014**, *3*, 53–56. [CrossRef]
48. Sanchez, B.; Bautista, J. Effects of furfural and 5-hydroxymethylfurfural on the fermentation of *Saccharomyces cerevisiae* and biomass production from *Candida guilliermondii*. *Enzyme Microb. Technol.* **1988**, *10*, 315–318. [CrossRef]
49. Ando, S.; Arai, I.; Kiyoto, K.; Hanai, S. Identification of aromatic monomers in steam-exploded poplar and their influences on ethanol fermentation by *Saccharomyces cerevisiae*. *J. Ferment. Technol.* **1986**, *64*, 567–570. [CrossRef]
50. Tran, A.V.; Chambers, R.P. Red oak wood derived inhibitors in the ethanol fermentation of xylose by *Pichia stipitis* CBS 5776. *Biotechnol. Lett.* **1985**, *7*, 841–845. [CrossRef]
51. Clark, T.A.; Mackie, K.L. Fermentation inhibitors in wood hydrolysates derived from the softwood *Pinus radiata*. *J. Chem. Technol. Biotechnol.* **1984**, *34*, 101–110. [CrossRef]
52. Erten, H. Relations between elevated temperatures and fermentation behaviour of *Kloeckera apiculata* and *Saccharomyces cerevisiae* associated with winemaking in mixed cultures. *World J. Microbiol. Biotechnol.* **2002**, *18*, 377–382. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.